

Watching proteins function with 150-ps time-resolved X-ray crystallography

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To gain a mechanistic understanding into how proteins execute their designed function, it is crucial to know how their structures evolve over time. To that end, we have developed the method of picosecond time-resolved macromolecular crystallography on the ID09B beamline at the European Synchrotron and Radiation Facility [1], and used this method to probe structural changes in myoglobin [2]. Myoglobin is a heme protein found in muscle that reversibly binds small ligands such as O₂, CO, and NO. Owing to the photosensitivity of the ligand bond and the reversibility of ligand binding, Mb has proven to be an excellent model system for investigating ligand migration and correlated structural changes in proteins. Picosecond laser pulses triggered CO dissociation from a ~250 micron P6 MbCO crystal, whose time-resolved diffraction pattern was recorded on a MAR CCD using time-delayed X-ray pulses generated by an in-vacuum undulator (~150-ps; ~10¹⁰ photons, 0.79 Å with a 3.5% bandwidth). The X-ray bandwidth was sufficient to obtain redundant data with images collected every 2 degrees. To obtain high-dynamic-range diffraction images with the available X-ray flux, approximately 8-32 X-ray shots were integrated on the MAR CCD before image readout. Because the protein crystal requires sufficient time to recover between photolysis pulses, which are intense enough to excite a significant fraction of the protein molecules, the maximum repetition rate used was 3.3 Hz. Diffraction images were accumulated with and without photolysis to generate accurate differences between the two diffraction data sets. To minimize systematic error in the time-resolved data, images were acquired at all time points before rotating the goniometer spindle. As many as 19 time points at 31 spindle orientations (19x31= 589 images) have been acquired from a single protein crystal.

High-resolution electron density maps of wild-type MbCO, shown in Fig. 1, reveal the order of events that accompany ligand translocation. Numerous features are observed including the displacement of the heme iron toward the proximal histidine, tilting of the heme, docking of CO in a site near the heme iron, and the correlated motion of protein side chains in the vicinity of the active binding site. Dramatic structural changes appear on the distal side of the heme, in particular the motion of the residues in the 29 and 64 positions. The Leu29 moves upward and the His64 shifts toward the site once occupied by CO, raising the barrier to geminate recombination. On the time scale of a few hundred ns, the “docked” CO slips around to the proximal side of the heme and is found in the so-called Xe1 docking site. On the microsecond time scale, H₂O manages to penetrate into the protein and is found at partial occupancy in the primary docking site. The structural changes that accompany ligand translocation help explain how the protein is able to excrete toxic CO with high efficiency, even though the CO is temporarily located so close to the active binding site. As demonstrated here, time-resolved crystallographic studies at 3rd generation synchrotrons can unveil at a high level of structural detail how a protein executes its function.

References

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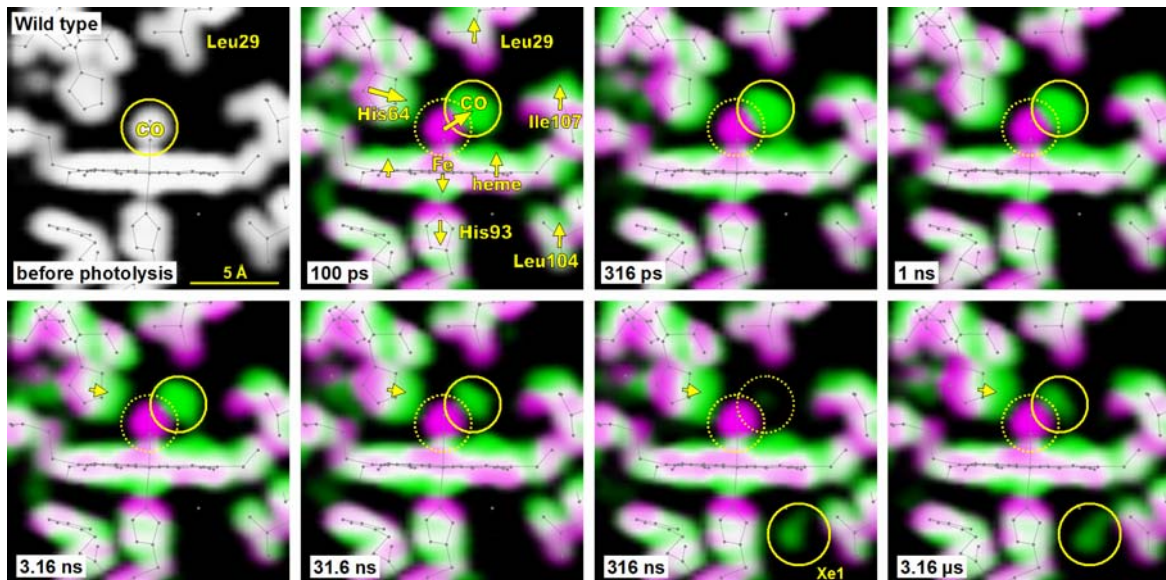


Figure 1. Time-resolved structural changes in wild-type MbCO are presented as a color-coded superposition of the ground state (magenta) and photolyzed state (green) electron density maps. Where magenta and green overlap, they blend to white. The direction of motion, as indicated by the arrows, follows the magenta-to-green color gradient. The solid circles denote occupied CO sites and the dotted circles denote evacuated CO sites. The photolyzed CO is initially trapped in the primary docking site about 2 Å from the binding site and subsequently migrates to the Xe1 site on the opposite side of the heme. Note the prompt movement of His64 toward the binding site in the 100 ps map. As CO escapes from the primary docking site, the color contrast across His64 increases, indicating further relaxation of that side chain. The grey stick model depicts the unphotolyzed structure, which provides a static reference frame for the time-resolved electron density changes.